

APPENDIX

IN THE SPECIFICATION:

Please amend the table on page 13 as follows:

Table 1 – Amino acid substitutions in the KNT mutant strains

| SEQ ID NO: | Residue No. | 2 17 | 25 | 57 | 61 | 62 | 66 | 75 | 91 | 94 | 102 1 | 12 1 | 16 | 117 | 159 | 188 1 | 190 ⁻ | 196 <i>′</i> | 197 | 198 | 199 2 | 203 2 | 206 2 | 207 2 | 211 2 | 220 2 | 234 2 | 238 2 | 246 |
|------------------|----------------|------|----|----|----|----|----|----|----|----|-------|------|----|-----|-----|-------|------------------|--------------|-----|-----|----------|-------|----------|-------|--|-------|---------|-----------|-----|
| 1 | WT* | NΗ | D | М | Ε | Α | Н | ٧ | Q | S | Q | S | L | Ε | T | S | S | V | Κ | Q | S | S | D | Н | F | S | L | T | D |
| <u>12</u> | KT3-1 | Υ | | | | | | Α | R | | R | Р | F | | | | | L | | | | | | | | | V | Α | |
| 13 | KT3-3 | | Ν | L | G | ٧ | | Α | | Р | R | | | G | | | L | | | | | | | | Ļ | | | | |
| 14 | KT3-5 | | | | G | | Υ | Α | R | Ρ | R | | | | | | | | | | Ρ | | | | | | V | | Ν |
| 15 | KT3-7 | S | | | G | | Υ | Α | R | | R | Ρ | F | | | | Τ | | | L | | | | | | | V | | |
| 2 | KT3-11 | K | | | G | | Υ | Α | R | | R | Р | F | | | | | | | | Р | | | | L | | | | |
| _ | KT3-12 | | | | | T | Υ | Α | R | Р | Κ | Τ | | | L | G | | | R | | Р | Р | | | | | | Α | |
| 17 | KT3-13 | | | | G | | Υ | Α | R | | R | Р | F | | | | | | | | P | | V | Q | | | | | |
| <u>18</u> | KT-3-15 | | | | G | | Υ | Α | R | | R | Р | F | | | | | | | | Р | | V | Q | | | | | |
| <u>19</u> | KT3-16 | | | | G | | Υ | Α | R | Р | Κ | Р | | | | | | | | | Р | | V | Q | | Р | | | |
| <u>20</u> | KT3-19 | | | L | | | Υ | Α | R | Р | K | Р | | | | | | | | | <u>P</u> | | <u> </u> | Q | | | | | |
| 3 | HTK | K | | L | G | ٧ | Υ | Α | R | Р | R | Р | F | | | | | | | | Р | Р | V_ | Q | <u> L </u> | P | <u></u> | <u> A</u> | |

IN THE CLAIMS:

1. A mutant kanamycin nucleotidyltransferase [having] comprising the sequence of SEQ ID NO:1 modified by at least one [or more] point mutation [mutations] selected from [a group consisting of] Met57Leu, [Ala62Val,] Ser94Pro, Ser203Pro, Asp206Val, His207Gln, Ser220Pro, Ile234Val and Thr238Ala [as against the protein comprising the amino acid sequence indicated by SEQ ID NO: 1], and having improved thermostability as compared to SEQ ID NO:1.

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PATENT Customer No. 22,852 Application No. 09/697,186 Attorney Docket No. 04853.0048-00

- 2. A mutant kanamycin nucleotidyltransferase with improved thermostability [, wherein it comprises] as compared to SEQ ID NO:1, comprising the amino acid sequence indicated by SEQ ID NO:2. [SEQ ID NO: 2.]
- 3. The <u>mutant</u> kanamycin nucleotidyltransferase according to claim 1, <u>comprising</u> [wherein it comprises] the amino acid sequence indicated [in SEQ ID NO: 3.] <u>by SEQ ID NO:3.</u>

IN THE ABSTRACT:

It is desirable to have [To obtain a] selective markers [marker] suitable for screening of thermophilic bacteria such as *Thermus thermophilus*. *T. thermophilus* are good research materials for investigating the interrelation between enzyme structures and functions since they are stable at extreme pH, crystallize easily and are easy-to-handle.

[To provide a novel] Novel mutants of Staphylococcus aureus kanamycin nucleotidyltransferase with markedly improved thermostability are disclosed, as well as a selective marker using the same, and a screening method for thermophilic bacteria such as [Thermus] <u>T.</u> thermophilus using said selective marker.

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Amino acid substitutions in the KN

Table

T mutant strains

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| 8 | z | | | | | × | | | 10 | | _ | ᅩ |
| Residue No. | *TW | KT3-1 | KT3-3 | KT3-5 | KT3-7 | KT3-11 | KT3-12 | KT3-13 | KT-3-15 | KT3-16 | KT3-19 | HTK |
| SEQ ID | - | 12 | 13 | 4 | 15 | 2 | 16 | 17 | 18 | 19 | 20 | က |

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mutation are mutually specific to each. Therefore, it is clear that these two KT3-15 has the same mis-sense mutation as KT3-13. These two mutants, share three silent mutations, however KT3-13's two silent mutations and KT3-15's one

mutants are distinct clones.